

Bile Acids Enhance Low Density Lipoprotein Receptor Gene Expression via a MAPK Cascade-mediated Stabilization of mRNA*

Received for publication, July 8, 2002, and in revised form, July 30, 2002
Published, JBC Papers in Press, July 30, 2002, DOI 10.1074/jbc.M206749200

Mayuko Nakahara, Hiroshi Fujii‡, Patrick R. Maloney§, Makoto Shimizu, and Ryuichiro Sato¶

From the Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, ‡Department of Signal Transduction Research, Niigata University, Graduate School of Medicine and Dental Sciences, Niigata 951-8510, Japan, and §Nuclear Receptor Discovery Research, GlaxoSmithKline, Research Triangle Park, North Carolina 27709

Recent studies have indicated that bile acids regulate the expression of several genes involved in bile acid and lipid metabolism as ligands for the farnesoid X receptor (FXR). We report here that bile acids are directly able to govern cholesterol metabolism by a novel mechanism. We show that chenodeoxycholic acid (CDCA) enhances low density lipoprotein (LDL) receptor gene expression in human cultured cell lines (HeLa, Hep G2, and Caco-2). The proteolytic activation of sterol regulatory element-binding protein-2 (SREBP-2), a major regulator for LDL receptor gene expression, is not affected by CDCA. Both deoxycholic acid and lithocholic acid as well as CDCA, but not ursodeoxycholic acid, increase the mRNA level for the LDL receptor, even when Hep G2 cells are cultured with 25-hydroxycholesterol, a potent suppressor of gene expression for the LDL receptor. Although it seems possible that FXR might be involved in genetic regulation, both reporter assays with a reporter gene containing the LDL receptor promoter as well as Northern blot analysis reveal that FXR is not involved in the process. On the other hand, inhibition of mitogen-activated protein (MAP) kinase activities, which are found to be induced by CDCA, abolishes the CDCA-mediated up-regulation of LDL receptor gene expression. We further demonstrate that CDCA stabilizes LDL receptor mRNA and that the MAP kinase inhibitors accelerate its turnover. Taken together, these results indicate that bile acids increase LDL uptake and the intracellular cholesterol levels through the activation of MAP kinase cascades in conjunction with a down-regulation of bile acid biosynthesis by FXR. This work opens up a new avenue for developing pharmaceutical interventions that lower plasma LDL by stabilizing LDL receptor mRNA.

Bile acids are synthesized from cholesterol in the liver and secreted as either taurine or glycine conjugates into bile. They are required for the efficient absorption of dietary fats and lipid-soluble vitamins in the gut. In humans, more than 90% of bile acids released into the duodenum are reabsorbed and returned to the liver, after which they are secreted again into bile. The catabolism of cholesterol to bile acid and their excretion are the main mechanisms for cholesterol elimination from

the body and thus play important roles in cholesterol homeostasis. Elevated concentrations of cholesterol within the liver promote bile acid synthesis through an increase in cholesterol 7 α -hydroxylase (CYP7a1) activity at the transcription level, the rate-limiting enzyme of the pathway. On the other hand, CYP7a1 expression is repressed by bile acids. Thus, CYP7a1 is under the influence of both feed forward and feedback regulation (1, 2).

CYP7a1 expression in rodents but not in humans (3, 4) is regulated by two nuclear receptors, the liver X receptor α (LXR α)¹ and FXR, both of which are abundantly expressed in the liver. LXR α is activated by cholesterol derivatives such as 24(S),25-epoxycholesterol and 24(S)-hydroxycholesterol and binds to a response element in the CYP7a1 promoter, thereby stimulating gene expression (5). FXR is activated by several different bile acids, including CDCA, as well as its glycine and taurine conjugates (6), and induces expression of the orphan receptor small heterodimer partner (SHP), an atypical member of the nuclear receptor family that lacks a DNA-binding domain, thereafter inhibiting the activity of the orphan nuclear hormone receptor liver receptor homologue, which stimulates CYP7a1 expression (7, 8).

Another cholesterol derivative, 25-hydroxycholesterol, is not active on LXR α but is a potent regulator for the membrane-bound transcription factors designated as SREBP, members of the basic helix-loop-helix leucine zipper family, which govern the transcription of genes for cholesterol synthesis enzymes as well as the LDL receptor (9–11). These proteins consist of ~1150 amino acids containing two transmembrane domains and the N-terminal regions, which are released by a two-step proteolytic processing in response to cholesterol depletion, thereafter being translocated to the nucleus where the active SREBPs induce the transcription of their responsive genes (12–15). Several different oxysterols and bile acids exert distinct effects on cholesterol and bile acid metabolism by regulating the activities of transcription factors and nuclear receptors.

Although it has been reported that the number as well as the genetic expression of the LDL receptor are up-regulated by CDCA (16–18), little is known about direct cross-talk between

* This work was supported by research grants from the Ministry of Education, Science, Sports, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed. Fax: 81-3-5841-8026; E-mail: aroysato@mail.ecc.u-tokyo.ac.jp.

¹ The abbreviations used are: LXR α , liver X receptor α ; FXR, farnesoid X receptor; CDCA, chenodeoxycholic acid; SHP, small heterodimer partner; LDL, low density lipoprotein; SREBP, sterol regulatory element-binding protein; MAP, mitogen-activated protein; LPDS, lipoprotein-deficient serum; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; PMA, phorbol 12-myristate 13-acetate; FBS, fetal bovine serum; RXR α , 9-*cis*-retinoic acid receptor α ; I-BABP, intestinal bile acid-binding protein; ERK, extracellular signal-regulated kinase; HMG, hydroxymethylglutaryl; PI3K, phosphatidylinositol 3-kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; UTR, untranslated region; ARE, AU-rich element.

bile acids and cholesterol metabolism. In the current report we have examined whether SREBPs are involved in CDCA-induced gene expression. Furthermore, based on sequence similarity between the 5'-flanking region of the human LDL receptor gene and a consensus sequence for FXR, an inverted repeat separated by 1 base pair (IR-1), we postulated that FXR might directly regulate transcription of the LDL receptor gene. To test this hypothesis, we have performed luciferase assays using a reporter gene containing the LDL receptor promoter in the presence of enforced-expressed FXR and CDCA and further investigated the effect of a potent, nonsteroidal FXR ligand. We demonstrate here that CDCA activates the MAP kinase cascade, but not FXR, thereby affecting the rapid degradation of LDL receptor mRNA.

EXPERIMENTAL PROCEDURES

Materials—CDCA, LY294002, wortmannin, actinomycin D, 25-hydroxycholesterol, and lipoprotein-deficient serum (LPDS) were purchased from Sigma. DCA, LCA, and UDCA were from Wako (Osaka, Japan). PD98059 and phorbol 12-myristate 13-acetate (PMA) were from Calbiochem and U0126 was from Promega.

Cell Culture—Hep G2, HeLa, and HEK293 cells were maintained in medium A (Dulbecco's modified Eagle's medium (Sigma), 100 units/ml penicillin, and 100 µg/ml streptomycin) supplemented with 10% fetal bovine serum (FBS) at 37 °C under 5% CO₂ atmosphere. Caco-2 cells were maintained in medium A supplemented with 10% FBS, 1% non-essential amino acids at 37 °C under 5% CO₂ atmosphere. The cells cultured for 10 days after total confluency were considered as differentiated.

Plasmid Constructs—Expression plasmids for human FXR and human 9-*cis*-retinoic acid receptor α (RXR α) were described previously (6). To generate pLDLR600, a 600-bp *Bam*HI-*Hind*III fragment (−595 to +36) containing an IR-1-like sequence (−338 to −350) from pLDLR (13) was ligated to the *Bgl*II-*Hind*III sites of a pGL3 basic vector (Promega). To generate pI-BABP, a *Bgl*II-*Hind*III PCR fragment coding the 5'-promoter region (−862/+30) of the human intestinal bile acid-binding protein (I-BABP) was inserted into a pGL3 basic vector.

Northern Blot Analysis—Total RNA was isolated using an RNA preparation kit (Isogen; Nippon Gene Corp.). The RNA was fractionated by electrophoresis in a 1% formaldehyde-agarose gel and transferred to nylon membranes (Hybond-N; Amersham Biosciences). Probes for human LDL receptor, SHP, microsomal triglyceride transfer protein, and 36B4 (19) were labeled with [α -³²P]dCTP (3000 Ci/mmol; Amersham Biosciences) using a random-primed DNA labeling kit (Megaprime DNA labeling system; Amersham Biosciences). The membrane was hybridized with radioactive cDNA probes, and the signals on the membrane were quantified using an image-analyzing system (FLA-3000; Fuji Film Inc.).

Antibodies—The polyclonal antibody (RS004) against human SREBP-2 (1–481) has been described previously (20). The polyclonal antibody against rat extracellular signal-regulated kinase (ERK, p42/44 MAP kinase) was purchased from Santa Cruz Biotechnology. The polyclonal anti-active ERK antibody was from Promega. Horseradish peroxidase-conjugated antibodies against rabbit immunoglobulins were from Amersham Biosciences.

Western Blot Analysis for SREBP-2—Hep G2 cells were harvested after 6 h of incubation with CDCA (250 µM) or after 48 h of incubation in the medium containing 5% LPDS supplemented with a 50 µM concentration of a hydroxymethylglutaryl (HMG)-CoA reductase inhibitor (pravastatin) plus 50 µM sodium mevalonate. Nuclear extracts were prepared as described (21). Proteins were fractionated by SDS/10% PAGE. Western blot analysis was carried out using anti-human SREBP-2 antibodies with chemiluminescent substrate (ECL; Amersham Biosciences).

Western Blot Analysis for ERK—Hep G2 cells were cultured with either CDCA (250 µM) or PMA (100 nM) for 4 h. U0126 was added 30 min prior to either CDCA or PMA. The cells were lysed in 50 mM Hepes pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 1 mM sodium vanadate, 50 mM sodium fluoride, and 20 mM β -glycerophosphate. Total cellular proteins were fractionated by SDS/10% PAGE. Western blot analysis was carried out using rabbit polyclonal antibodies against either ERK or the dually phosphorylated active form of ERK with chemiluminescent substrate.

Dual Luciferase Assay—HEK293 cells (35-mm dishes) were transfected by the calcium phosphate method with 0.2 µg of a reporter

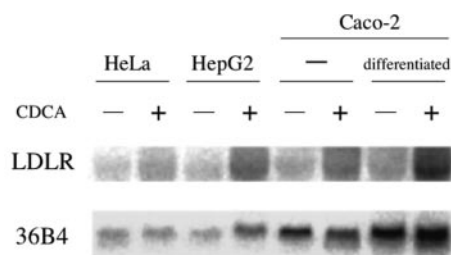


FIG. 1. **Effect of CDCA on LDL receptor gene expression in human cultured cell lines.** HeLa, Hep G2, and Caco-2 (undifferentiated or differentiated) cells were cultured with 250 µM CDCA for 24 h. Total RNA (20 µg/lane) was subjected to electrophoresis and blot hybridization with the ³²P-labeled probe for either the LDL receptor (*LDLR*) or 36B4.

plasmid, 0.01 µg of pRL-SV40 (Promega), and expression plasmids (0.1 µg each) for human FXR and human RXR α (6). Forty-eight hours later both firefly and renilla luciferase activities were quantified using a dual luciferase reporter system (Promega) according to the manufacturer's instructions (21).

RESULTS

CDCA Induces LDL Receptor Gene Expression in Human Cultured Cells—Several investigators have reported that CDCA induces the LDL receptor expression in Hep G2 cells (16–18). To test whether this induction is observed in nonhepatic cells, Caco-2 cells, which share the morphological and functional properties of the ileal enterocytes, together with HeLa cells were cultured with 250 µM CDCA for 24 h, and total RNA was extracted. Because we observed that I-BABP gene expression was induced more by CDCA in differentiated Caco-2 cells than in undifferentiated Caco-2 cells (data not shown), RNA from both undifferentiated and differentiated Caco-2 cells was also prepared to study the difference in the CDCA-mediated induction related to stage of differentiation. Northern blot analyses using an LDL receptor and a control 36B4 probe were carried out (Fig. 1). In all cells the LDL receptor mRNA levels were up-regulated ~2-fold by CDCA, indicating that induction is not restricted to Hep G2 cells. In Caco-2 cells the stage of differentiation, with which expression of various genes dramatically changes, did not affect CDCA-mediated induction.

SREBP-2 Is Not Activated by CDCA in Hep G2 Cells—Although the effect of CDCA was observed in all cells tested, we used Hep G2 cells in the following experiments to elucidate the mechanism of CDCA-induced expression of the LDL receptor gene in the liver, the primary organ for cholesterol and bile acid homeostasis. The results in Fig. 1 led us to speculate that SREBPs, which mainly regulate LDL receptor gene expression in response to the intracellular cholesterol level, might be activated by CDCA. To test this possibility, Hep G2 cells were cultured with a medium containing 10% fetal calf serum and 0.3 µg/ml 25-hydroxycholesterol (a potent oxysterol included to reduce the amount of nuclear SREBPs), either in the absence or presence of CDCA. As a control, Hep G2 cells were cultured with a medium containing an HMG-CoA reductase inhibitor to induce the amounts of nuclear SREBPs as well as the LDL receptor mRNA level. Nuclear extracts were prepared and Western blot analyses were performed using an anti-human SREBP-2 antibody (Fig. 2A). The amount of nuclear SREBP-2 was reduced by the addition of 25-hydroxycholesterol (lane 1) but was unaltered by treatment with CDCA (lane 2), whereas depletion of cellular cholesterol brought about a marked increase in the nuclear SREBP-2 (lane 3). Fig. 2B shows that CDCA augmented the LDL receptor mRNA level even in the presence of 25-hydroxycholesterol (lane 2). These results clearly indicate that nuclear SREBP-2 alone cannot account for the CDCA-induced expression of the LDL receptor gene. It

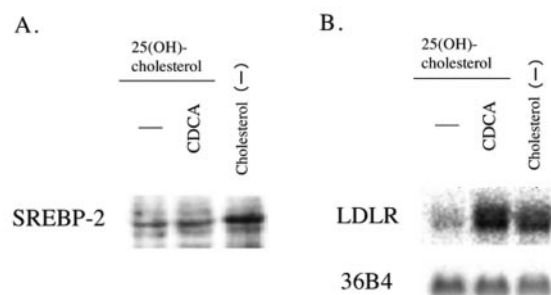


FIG. 2. **The amounts of nuclear SREBP-2 in Hep G2 cells.** Hep G2 cells were cultured in a medium containing 10% FBS plus 0.3 $\mu\text{g}/\text{ml}$ 25-hydroxycholesterol (25(OH)cholesterol) with or without 250 μM CDCA for 6 h, or cultured in a medium containing 5% LPDS supplemented with a 50 μM concentration of an HMG-CoA reductase inhibitor (pravastatin) plus 50 μM sodium mevalonate (cholesterol(-)) for 48 h. A, aliquots of the nuclear extract protein (38 $\mu\text{g}/\text{lane}$) were subjected to SDS-PAGE and Western blotting using an anti-SREBP-2 antibody. B, total RNA (15 $\mu\text{g}/\text{lane}$) was subjected to electrophoresis and blot hybridization with the indicated ^{32}P -labeled probe. In three separate experiments the same results were obtained. LDLR, LDL receptor.

should be noted that the effect of CDCA overcomes the suppressive effect of 25-hydroxycholesterol on LDL receptor gene expression.

Both DCA and LCA as Well as CDCA, but Not UDCA, Induce LDL Receptor Gene Expression—We next investigated whether other bile acid molecules also affect gene expression in Hep G2 cells. In the following experiments the cells were cultured with 25-hydroxycholesterol to suppress LDL receptor mRNA, bringing about a situation in which the induction by bile acids would be clear. Because a dose of more than 100 μM CDCA under these conditions significantly and dose dependently induces LDL receptor mRNA (data not shown), a 250 μM concentration of bile acids was utilized in the current experiments. As shown in Fig. 3, CDCA, DCA, and LCA, but not UDCA, significantly induced the LDL receptor mRNA level, and a similar pattern was observed on regulation of SHP gene expression, which is one of the FXR-responsive genes in the liver. The only minor difference between the two patterns is that LCA, which has been reported to be a weak activator of FXR, only slightly induced the SHP mRNA level, whereas LDL receptor gene expression was significantly enhanced.

FXR Is Not Involved in the Transcriptional Regulation of the LDL Receptor Gene—The above data suggest that the LDL receptor gene might be one of the FXR-responsive genes. To evaluate the involvement of FXR in the up-regulation, we performed luciferase assays using reporter genes containing either the promoter region of the human LDL receptor or the human I-BABP gene, which is one of the FXR-responsive genes. The cells were cultured with 100 μM CDCA for 2 days because the concentration of 250 μM caused severe toxicity in the cells subjected to DNA transfection. When HEK293 cells were transfected with expression plasmids for both FXR and RXR, CDCA significantly induced luciferase activities driven by the I-BABP promoter (Fig. 4A). On the other hand, the LDL receptor gene expression, which was significantly induced by cholesterol depletion (Fig. 4A, lower panel), was only slightly stimulated by CDCA in the presence or absence of FXR/RXR (Fig. 4A, upper panel). To further confirm the dissociation of FXR from the CDCA-induced regulation, Hep G2 cells were cultured with either CDCA or GW4064, a synthetic FXR ligand (7), for 6 h, and induction of the LDL receptor and SHP mRNA was analyzed. As shown in Fig. 4B, the agonist did not mimic the stimulating effect of CDCA on LDL receptor gene expression, whereas both ligands significantly enhanced SHP gene expression. These results strongly support the notion that CDCA

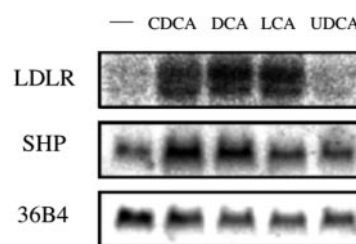


FIG. 3. **Effect of different bile acids on the expression of LDL receptor (LDLR) gene in Hep G2 cells.** Hep G2 cells were cultured with 0.3 $\mu\text{g}/\text{ml}$ 25-hydroxycholesterol plus 250 μM indicated bile acids for 6 h. Total RNA (15 $\mu\text{g}/\text{lane}$) was subjected to electrophoresis and blot hybridization with the indicated ^{32}P -labeled probe.

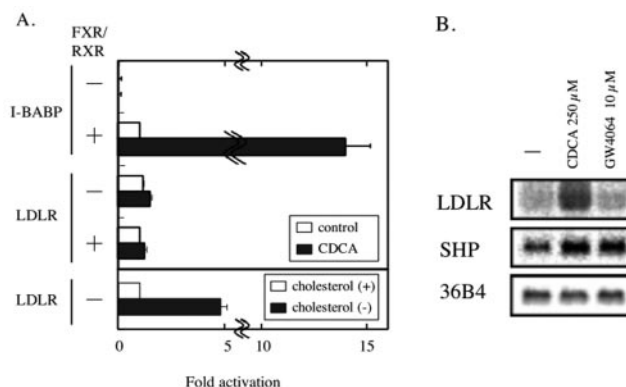


FIG. 4. **Effects of FXR on luciferase activities of the LDL receptor promoter-containing reporter gene and the expression of LDL receptor gene.** A, HEK293 cells were cotransfected with either pI-BABP or pLDLR600 together with pRL-SV40 and expression plasmids for human FXR and RXR α . The cells were incubated with a medium containing 10% charcoal-stripped FBS, 0.3 $\mu\text{g}/\text{ml}$ 25-hydroxycholesterol, and 100 μM CDCA for 48 h, and then luciferase assays were performed as described under "Materials and Methods." Luciferase activities in the presence of FXR/RXR without CDCA are considered as 1. The cells transfected with pLDLR600 were incubated with a medium containing 5% LPDS supplemented with either 1 $\mu\text{g}/\text{ml}$ 25-hydroxycholesterol plus 10 $\mu\text{g}/\text{ml}$ cholesterol (cholesterol(+)) or a 50 μM concentration of an HMG-CoA reductase inhibitor plus 50 μM sodium mevalonate (cholesterol(-)). Luciferase activities under cholesterol-loaded conditions are considered as 1. The values given are the average of data from three experiments. Data are expressed as means \pm S.D. B, Hep G2 cells were cultured with 0.3 $\mu\text{g}/\text{ml}$ 25-hydroxycholesterol plus 250 μM CDCA or 10 μM GW4064 for 6 h. Total RNA (15 $\mu\text{g}/\text{lane}$) was subjected to electrophoresis and blot hybridization with the indicated ^{32}P -labeled probe. LDLR, LDL receptor.

induces LDL receptor gene expression by a mechanism distinct from that of the FXR-mediated transcriptional up-regulation.

MAP Kinase Inhibitors, but Not Phosphatidylinositol 3-Kinase (PI3K) Inhibitors, Abolish CDCA-mediated Regulation—Accumulating evidence suggests that bile acids modulate signal transduction pathways including the protein kinase A, protein kinase C-, MAP kinase-, c-Jun N-terminal kinase- and PI3K-dependent cascades (22–25). To determine which of these pathways is associated with the regulation of the gene expression in these experiments, the effects of various inhibitors on the CDCA-induced expression were investigated. Treatment of cells with one of the PI3K inhibitors, wortmannin or LY294002, had no effect on the CDCA-mediated up-regulation of LDL receptor gene expression (Fig. 5A). On the contrary, Fig. 5B shows that the MAP kinase/ERK kinase (MEK) 1/2 inhibitors U0126 and PD98059 led to significant reduction of the LDL receptor mRNA levels induced by CDCA. These results indicate that the MEK/ERK pathway mainly regulates the gene expression of the LDL receptor.

CDCA Activates ERK 1/2 in Hep G2 Cells—To confirm activation of ERK 1/2 by CDCA, we subjected cell extracts from

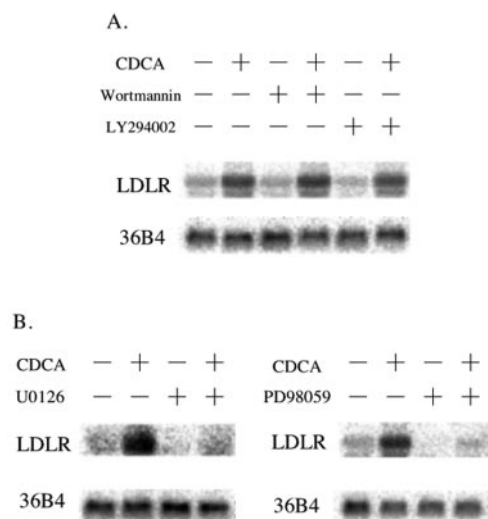


FIG. 5. Effects of PI3K and MEK inhibitors on the up-regulation of LDL receptor gene by CDCA. After preincubation with the indicated inhibitor (A, 250 nM wortmannin, 50 μ M LY294002; B, 20 μ M U0126, 20 μ M PD98059) for 30 min, 250 μ M CDCA plus 0.3 μ g/ml 25-hydroxycholesterol were added to the medium, and Hep G2 cells were incubated for 6 h. Total RNA (15 μ g/lane) was subjected to electrophoresis and blot hybridization with the indicated 32 P-labeled probe. In three separate experiments the same results were obtained. *LDLR*, LDL receptor.

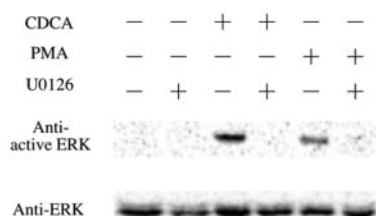


FIG. 6. Effect of CDCA on activation of ERK. Hep G2 cells were cultured with either 250 μ M CDCA or 100 nM PMA for 4 h. U0126 (20 μ M) was added 30 min prior to either CDCA or PMA. Total cellular protein (50 μ g/lane) was subjected to SDS-PAGE and Western blotting using antibodies specific for either the activated, phosphorylated forms of ERK 1/2 (the upper panel) or all forms, nonphosphorylated and phosphorylated, of ERK 1/2 (the lower panel).

CDCA-treated cells to Western blot analysis with an antibody that specifically recognizes the activated, phosphorylated forms of ERK 1/2 (Fig. 6). Hep G2 cells were also treated with PMA to activate the MEK/ERK pathway (26). The amounts of phosphorylated ERK 1/2 were very low in control cells but increased markedly in CDCA- and PMA-treated cells (lanes 1, 3, and 5). Treatment of these cells with U0126 significantly inhibited both CDCA- and PMA-mediated activation of ERK (lanes 4 and 6). U0126 did not affect ERK protein expression, as indicated by Western blotting of the same cell extracts with an anti-ERK 1/2 antibody. Thus, CDCA activates ERK 1/2 via MEK activation, which can be inhibited by U0126. The same results were obtained in PD98059-treated cells (data not shown).

CDCA Prolongs the Half-life of LDL Receptor mRNA—We next examined whether CDCA stabilizes LDL receptor mRNA, thereby inducing the mRNA levels. Hep G2 cells were pre-treated with actinomycin D for 30 min and then further treated with or without CDCA for the indicated period. As shown in Fig. 7, the turnover rate of LDL receptor mRNA was prolonged 2-fold ($t_{1/2} = 3$ versus 6 h) by CDCA. The turnover of microsomal triglyceride transfer protein mRNA, which is involved in lipoprotein synthesis, was not affected by CDCA.

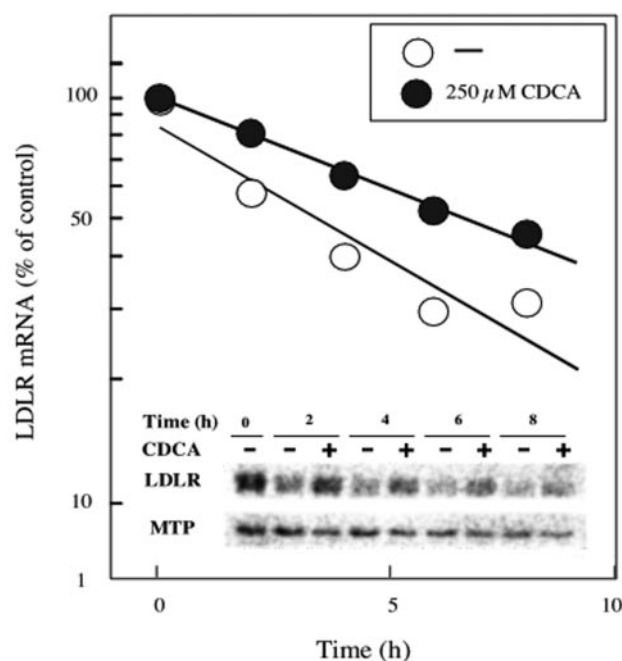


FIG. 7. Effect of CDCA on stability of LDL receptor (*LDLR*) mRNA. Hep G2 cells were incubated with a medium containing 5% LPDS for 48 h. After preincubation with 5 μ g/ml actinomycin D for 30 min, 250 μ M CDCA was added to the medium, and the cells were incubated for the indicated time. Total RNA (15 μ g/lane) was subjected to electrophoresis and blot hybridization with the indicated 32 P-labeled probe. The signals on the membrane were quantified, and data were plotted as the percentage of the LDL receptor mRNA remaining. In three separate experiments the same results were obtained. *MTP*, microsomal triglyceride transfer protein.

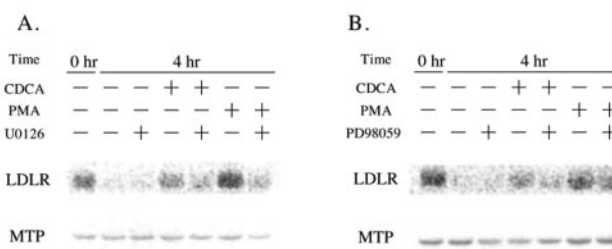


FIG. 8. Effect of MEK inhibitors on enhanced stability of LDL receptor (*LDLR*) mRNA by CDCA. Hep G2 cells were incubated with a medium containing 5% LPDS for 48 h. After preincubation with 5 μ g/ml actinomycin D plus either 20 μ M U0126 (A) or 20 μ M PD98059 (B) for 30 min, either 250 μ M CDCA or 100 nM PMA was added to the medium, and the cells were incubated for 4 h. Total RNA (15 μ g/lane) was subjected to electrophoresis and blot hybridization with the indicated 32 P-labeled probe. *MTP*, microsomal triglyceride transfer protein.

We further determined whether CDCA-mediated stabilization of LDL receptor mRNA depended on activation of the MAP kinase pathway. Hep G2 cells were incubated with either CDCA or PMA in the presence or absence of a MEK inhibitor, and mRNA decay for 4 h was examined (Fig. 8, A and B). Treatment of cells with PMA as well as CDCA stabilized LDL receptor mRNA. The MEK inhibitors, U0126 and PD98059, blocked CDCA- or PMA-mediated stabilization of LDL receptor mRNA. These results suggest that the MEK/ERK pathway is associated with stabilization and that this effect can account for the CDCA-induced expression of LDL receptor gene.

DISCUSSION

In the current report we provide the first evidence that bile acids, the final products of the cholesterol-bile acid biosynthetic

pathway in the liver, directly regulate the LDL receptor gene expression by a novel mechanism. It is well established that bile acids undergoing enterohepatic circulation feedback repress their own biosynthesis. The activity of the first and rate-limiting enzyme in the biosynthetic pathway, CYP7a1, is repressed at the level of gene transcription, which effect is exerted by the bile acid-activated FXR. Based on sequence similarity between the human LDL receptor promoter -338 to -350 (5'-AGGACAaTGGCAT-3') and IR-1 (5'-AGGTCAaTGACCT-3'), a consensus sequence for FXR, we initially hypothesized that the gene is directly regulated by FXR. However, we here have reported that bile acids stabilize and increase LDL receptor mRNA through activation of the MAP kinase cascade. Therefore, it is evident that bile acids dynamically control the hepatic cholesterol levels through two distinct pathways and mechanisms, the repression of conversion from cholesterol to bile acids and also the induction of the LDL receptor.

It has been reported that transient activation of the MAP kinase cascade by PMA or cytokines up-regulates LDL receptor transcription in an SREBP-independent manner (26–29). Gel shift assays using nuclear extracts prepared from untreated and the cytokine oncostatin M-treated Hep G2 cells revealed that an as yet unidentified complex, which appears with the administration of oncostatin M and disappears with U0126, is bound to the sterol-independent regulatory element in the LDL receptor promoter region -17 to -1 (30). Furthermore, it has been demonstrated that MAP kinases phosphorylate both SREBPs and Sp1, which coordinately regulate LDL receptor gene expression, and augment their transcriptional activities (31–33). We found that CDCA as well as PMA indeed activates the MAP kinase cascade (Fig. 6). Therefore, it is likely that the CDCA-dependent increase in LDL receptor mRNA observed in Figs. 1–5 is in part because of a transcriptional up-regulation. In addition, the slight decrease of LDL receptor mRNA by treatment of the MAP kinase inhibitor alone (Fig. 5B) suggests that MAP kinase activity might be involved in the basal transcription of LDL receptor gene. The current results and previous findings taken together strongly support the notion that CDCA activates the MAP kinase cascade, thereby stimulating the LDL receptor gene expression at the transcriptional as well as the posttranscriptional level.

The mechanism by which CDCA stabilizes the LDL receptor mRNA remains unresolved. It has been reported that the 3'-untranslated region (UTR) of human LDL receptor mRNA contains three AU-rich elements (AREs) based on sequence homology with the nonameric sequence UUAUUUAU and that the sequence is sufficient to confer a brief constitutive mRNA half-life (34). Indeed, the human LDL receptor expression was found to be up-regulated by enhanced stability of the mRNA in mice engineered to express human LDL receptor, lacking the 3'-UTR sequence, instead of the mouse LDL receptor (35). Furthermore, three *alu*-like repetitive elements in the distal 3'-UTR have been shown to confer stability to the mRNA in the presence of PMA (34). It is also reported that cyclooxygenase-2 mRNA is stabilized by a MEK-dependent mechanism (36). Although it remains unclear whether the PMA-induced stabilization is directly associated with the MEK/ERK activation, these observations support the general notion that the MAP kinase cascades are involved in stabilization of certain mRNA. A recent study demonstrated that the mammalian exosome interacts with the ARE-binding protein, thereby promoting the rapid degradation of ARE-containing RNA (37). It is, therefore, possible that the *alu*-like repetitive elements do play an important role in the ARE-containing RNA degradation by the exosome in the presence of either PMA or CDCA. Whether in fact this is the case, and if so, the mechanism by which the 3'-UTR

sequence of LDL receptor mRNA is involved in the CDCA-induced stability are under active investigation.

We observed that UDCA from among the bile acids tested in the current experiments had the least effect on the LDL receptor and SHP gene expression (Fig. 3). It seems likely that the structural difference between CDCA and UDCA, specifically the 7 β -hydroxy epimer of CDCA, is critical for the activation of the MAP kinases as well as for the recognition of the ligand for FXR (38, 39). It is also noted that LCA, a weak activator of FXR, is a quite potent inducer of LDL receptor gene expression. Indeed, LCA was found to be more potent than cholic acid, and UDCA had no effect on the activation of MAP kinases (25). Because the non-ionic non-cytolytic membrane detergent octyl β -D-glucopyranoside had no effect on MAP kinase activation (40), these bile acids appear to induce the activities of MAP kinases in a specific manner, not by their detergent-like effects.

The observation that the CDCA-mediated elevation of LDL receptor mRNA even in the presence of 25-hydroxycholesterol was comparable with induction in response to cholesterol depletion (Fig. 2B) suggests that stabilization of LDL receptor mRNA may be a critical target for the modulation of human lipid metabolism. Ultimately, it may be possible to identify desirable compounds that have selective activity on stabilization of mRNA for the LDL receptor, thereby reducing the risk for atherosclerosis.

Acknowledgments—We thank Dr. Timothy M. Willson (GlaxoSmith-Kline) and Dr. Yasuhisa Fukui (University of Tokyo) for helpful suggestions. We thank Dr. Kevin Boru for review of the manuscript.

REFERENCES

- Kliwer, S. A., Lehmann, J. M., and Willson, T. M. (1999) *Science* **284**, 757–760
- Russell, D. W. (1999) *Cell* **97**, 539–542
- Chen, J., Cooper, A. D., and Levy-Wilson, B. (1999) *Biochem. Biophys. Res. Commun.* **260**, 829–834
- Agellon, L. B., Drover, V. A. B., Cheema, S. K., Gbaguidi, G. F., and Walsh, A. (2002) *J. Biol. Chem.* **277**, 20131–20134
- Lehmann, J. M., Kliwer, S. A., Moore, L. B., Smith-Oliver, T. A., Oliver, B. B., Su, J.-L., Sundseth, S. S., Winegar, D. A., Blanchard, D. E., Spencer, T. A., and Willson, T. M. (1997) *J. Biol. Chem.* **272**, 3137–3140
- Grober, J., Zaghini, I., Fujii, H., Jones, S. A., Kliwer, S. A., Willson, T. M., Ono, T., and Besnard, P. (1999) *J. Biol. Chem.* **274**, 29749–29754
- Goodwin, B., Jones, S. A., Price, R. R., Watson, M. A., McKee, D. D., Moore, L. B., Galarzi, C., Willson, J. G., Lewis, M. C., Roth, M. E., Maloney, P. R., Willson, T. M., and Kliwer, S. A. (2000) *Mol. Cell* **6**, 517–526
- Lu, T. T., Makishima, M., Repa, J. J., Schoonjans, K., Kerr, T. A., Auwerx, J., and Mangelsdorf, D. J. (2000) *Mol. Cell* **6**, 507–515
- Brown, B. S., and Goldstein, J. L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 11041–11048
- Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L., and Brown, M. S. (1993) *Cell* **75**, 187–197
- Hua, X., Yokoyama, C., Wu, J., Briggs, M. R., Brown, M. S., Goldstein, J. L., and Wang, X. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 11603–11607
- Sato, R., Yang, J., Wang, X., Evans, M. J., Ho, Y. K., Goldstein, J. L., and Brown, M. S. (1994) *J. Biol. Chem.* **269**, 17267–17273
- Sato, R., Inoue, J., Kawabe, Y., Kodama, T., Takano, T., and Maeda, M. (1996) *J. Biol. Chem.* **271**, 26461–26464
- Inoue, J., Sato, R., and Maeda, M. (1998) *J. Biochem. (Tokyo)* **123**, 1191–1198
- Sato, R., Okamoto, A., Inoue, J., Miyamoto, W., Sakai, Y., Emoto, N., Shimano, H., and Maeda, M. (2000) *J. Biol. Chem.* **275**, 12497–12502
- Taniguchi, T., Chen, J., and Cooper, A. D. (1994) *J. Biol. Chem.* **269**, 10071–10078
- Carlson, T. L., and Kottke, B. A. (1989) *Biochem. J.* **264**, 241–247
- Kawabe, Y., Shimokawa, T., Matsumoto, A., Honda, M., Wada, Y., Yazaki, Y., Endo, A., Itakura, H., and Kodama, T. (1995) *Biochem. Biophys. Res. Commun.* **208**, 405–411
- Inoue, J., Kumagai, H., Terada, T., Maeda, M., Shimizu, M., and Sato, R. (2001) *Biochem. Biophys. Res. Commun.* **283**, 1157–1161
- Sato, R., Miyamoto, W., Inoue, J., Terada, T., Imanaka, T., and Maeda, M. (1999) *J. Biol. Chem.* **274**, 24714–24720
- Hirano, Y., Yoshida, M., Shimizu, M., and Sato, R. (2001) *J. Biol. Chem.* **276**, 36431–36437
- Bouscarel, B., Kroll, S. D., and Fromm, H. (1999) *Gastroenterology* **117**, 433–452
- Gupta, S., Stravitz, R. T., Dent, P., and Hylemon, P. B. (2001) *J. Biol. Chem.* **276**, 15816–15822
- Rust, C., Karnitz, L. M., Paya, C. V., Moscat, J., Simari, R. D., and Gores, G. J. (2000) *J. Biol. Chem.* **275**, 20210–20216
- Brady, L. M., Beno, D. W. A., and Davis, B. H. (1996) *Biochem. J.* **316**, 765–769
- Kumar, A., Chambers, T. C., Cloud-Heflin, B. A., and Mehta, K. D. (1997) *J. Lipid Res.* **38**, 2240–2248
- Kumar, A., Middleton, A., Chambers, T. C., and Mehta, K. D. (1998) *J. Biol.*

- Chem.* **273**, 15742–15748
28. Li, C., Kraemer, F. B., Ahlborn, T. E., and Liu, J. (1999) *J. Biol. Chem.* **274**, 6747–6753
29. Maker, R. S. J., Lipsky, P. E., and Cuthbert, J. A. (2000) *J. Lipid Res.* **41**, 762–774
30. Liu, J., Ahlborn, T. E., Briggs, M. R., and Kraemer, F. B. (2000) *J. Biol. Chem.* **275**, 5214–5221
31. Kotzka, J., Muller-Wieland, D., Roth, G., Kremer, L., Munck, M., Schurmann, S., Knebel, B., and Krone, W. (2000) *J. Lipid Res.* **41**, 99–108
32. Roth, G., Kotzka, J., Kremer, L., Lehr, S., Lohaus, C., Meyer, H. E., Krone, W., and Muller-Wieland, D. (2000) *J. Biol. Chem.* **275**, 33302–33307
33. Milanini-Mongiati, J., Pouyssegur, J., and Pages, G. (2002) *J. Biol. Chem.* **277**, 20631–20639
34. Wilson, G. M., Vasa, M. Z., and Deeley, R. G. (1998) *J. Lipid Res.* **39**, 1025–1032
35. Knouff, C., Malloy, S., Wilder, J., Altenburg, M. K., and Maeda, N. (2001) *J. Biol. Chem.* **276**, 3856–3862
36. Xu, K., Robida, A. M., and Murphy, T. J. (2000) *J. Biol. Chem.* **275**, 23012–23019
37. Chen, C-Y., Gherzi, R., Ong, S-E., Chan, E. L., Rajmakers, R., Pruijn, G. J. M., Stoecklin, G., Moroni, C., Mann, M., and Karin, M. (2001) *Cell* **107**, 451–464
38. Makishima, M., Okamoto, A. Y., Repa, J. J., Tu, H., Learned, R. M., Luk, A., Hull, M. V., Lustig, K. D., Mangelsdorf, D. J., and Shan, B. (1999) *Science* **284**, 1362–1365
39. Parks, D. J., Blanchard, S. G., Bledsoe, R. K., Chandra, G., Consler, T. G., Kliewer, S. A., Stimmel, J. B., Willson, T. M., Zavacki, A. M., Moore, D. D., and Lehmann, J. M. (1999) *Science* **284**, 1365–1368
40. Marriott, S. J., Griffith, G. R., and Consigli, R. A. (1987) *J. Virol.* **61**, 375–382

**Bile Acids Enhance Low Density Lipoprotein Receptor Gene Expression via a
MAPK Cascade-mediated Stabilization of mRNA**
Mayuko Nakahara, Hiroshi Fujii, Patrick R. Maloney, Makoto Shimizu and Ryuichiro
Sato

J. Biol. Chem. 2002, 277:37229-37234.

doi: 10.1074/jbc.M206749200 originally published online July 30, 2002

Access the most updated version of this article at doi: [10.1074/jbc.M206749200](https://doi.org/10.1074/jbc.M206749200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 40 references, 30 of which can be accessed free at
<http://www.jbc.org/content/277/40/37229.full.html#ref-list-1>